

Fear and Aggression in Large Flocks of Laying Hens

Effects of sex composition

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Love is all you need

*J. Lennon
(1967)*

Abstract

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The role of the male in wild flocks of fowl, is to supervise the flock and guard it against predators, probably thereby lowering aggression and fearfulness among the members of the flock. The aim of this thesis was to study the influence of males on female behaviour in commercial farm situations by comparing all-female to sex-mixed groups of laying hens in large flocks.

It was shown that males had a reducing effect on female aggression in large of laying hens (*Gallus gallus domesticus*) housed in groups of approx. 500 birds at a sex ratio of 1male: 24 females in a high density aviary system. However, no effect on aggression was found in flocks (ranging in size from 250 to 5000 birds) in two different loose housing systems on 25 commercial farms, probably due to the low number of males in the flocks (~ 1:350).

Experiments showed that female birds in mixed flocks roosting closely together were less aggressive towards each other than birds roosting far apart. This indicates recognition of roosting partners as well as an effect of the males. Irrespective of sex composition, females in large groups were highly constant in their use of space if they were marked roosting in the ends of the compartments.

Also fear reactions in females were reduced by the presence of males, as studied in large groups of 1200 birds with a sex ratio of 1:100. The vigilance behaviour and duration of tonic immobility were used as indicators, as these behaviours are considered to be protective behaviours in relation to predators.

In a study of groups of 1750 birds at a sex ratio of 1:190, a positive correlation between male rank order and area covered was found. Most males, though, used more than half of the available area. However no female attachment to specific males was observed.

In conclusion, males reduce female aggression and fearfulness also in large flocks and their influence on aggression is probably mainly through direct social dominance. Further, for subgroup formation in large flocks to occur, environmental features that facilitate localisation may be important.

Key words: agonistic behaviour, cockerels, feather pecking, layers, males, roosters, space use, subgroups, tonic immobility, vigilance

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Appendix

Papers I -V

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-V):

- I. Odén, K., Vestergaard, K.S & Algers, B. 1999. Agonistic behaviour and feather pecking in single-sexed and mixed groups of laying hens. *Applied Animal Behaviour Science*, 62: 219-230.
- II. Odén, K., Vestergaard, K.S. & Algers, B. 2000. Space use and agonistic behaviour in relation to sex composition in large flocks of laying hens. *Applied Animal Behaviour Science*, 67 (4): 307-320.
- III. Odén, K., Keeling, L.J. & Algers, B. 2002. Behaviour of laying hens in two types of aviary systems on 25 commercial farms in Sweden. *British Poultry Science* 43: 169-181.
- IV. Odén, K., Gunnarsson, S., Berg, C. & Algers, B. Effects of sex composition on fear measured as tonic immobility and vigilance behaviour in large flocks of laying hens. *Submitted*.
- V. Odén, K., Berg, C., Gunnarsson, S. & Algers, B. Male rank order, space use and female attachment in large flocks of laying hens. *Submitted*.

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Introduction

Behaviour of fowl

Red jungle fowl (*Gallus gallus*), the ancestor of the domesticated fowl, form, like feral domestic fowl, hierarchical harem groups which stay within a certain area, the home range or territory when defended during the breeding season by the dominant male. If undisturbed, the group also have a constant roosting site (Collias and Collias, 1967). Females and males have separate dominance or peck orders, where males usually are dominant over females. The peck orders are typically linear, but in larger groups the hierarchy is mostly more complex, as described already in 1922 by Schjelderup-Ebbe followed by many authors. The rooster plays a leading role in the hierarchy, fertilises the females and guards the group against predators (McBride et al., 1969; Johnson, 1963; Sullivan, 1991).

Anti-predator behaviour

In prey animals like fowl specific protection behaviours have evolved, like the habit of roosting high up at night to avoid hunters on the ground; searching for food in areas protected from aerial predators; being nearly constantly vigilant and reacting upon alarm signals from conspecifics (Collias, 1987). Fowl, like other birds, depend heavily on their hearing and sight capacities, which are well developed. A low threshold for signals resulting in quick escape behaviour, probably has a high fitness value in evolutionary terms (Rovee-Collier et al., 1983). Likewise, if being held by the claws or jaws of a predator, the last chance may be to "play dead" and make the aggressor relax long enough to get the opportunity to escape. This has probably resulted in a high evolutionary pressure for the tendency to death feign or show tonic immobility, which is the scientific term (Gallup 1977).

Aggression

The theories of populations spreading as a result of increased social encounters when the number of individuals rise are well known in the field of experimental biology (Brown, 1975). Adverse effects of crowding, with increased agonistic behaviour, have also been observed under laboratory conditions for example in rodents (Christian, 1970). When resources are scarce and there is competition for them, there is nearly always a corresponding high

level of aggression (Christian, 1970). A high frequency of aggressive behaviour may also reflect social instability in the group as the peck-order is broken or unacquainted birds meet within a large flock. One might therefore expect increased aggression in larger flocks compared to small ones. In a large flock a hen cannot recognize all other individuals. Recognition occurs apparently through vision (Guhl and Ortman, 1953) and the maximum size of the recognizable group is still not known, but it is probably not larger than about 100 other individuals (Guhl, 1953).

Males and territories

Males - even subordinate ones - have been reported to have a kind of portable territory (McBride et al., 1969). According to McBride and co-workers (1969) territorial behaviour or personal spheres are not, however, uniquely male phenomena, as broody hens also defend the area around their chicks. Craig and Guhl (1969) found territorial behaviour in female flocks of domestic fowl (Rhode Island Reds). They also found that the tendency for individual birds to stay and be dominant within a certain area seemed to be stronger in flocks of 400 than in flocks of 200 birds. For a rooster, however, it is natural to gather hens and guard them against predators as well as other males (McBride et al., 1969).

The rooster in the history of man

The courage and splendour of the rooster has fascinated man through history. He has been a symbolic animal in many cultures. In ancient Egypt he was looked upon as a reflex of the sun and protector of the dead. In the Nordic mythology he kept watch high up in the tree Yggdrasil; At the Twilight of the Gods he would awake Oden's warriors in Valhalla. In Christianity he was portending the birth and the resurrection of Christ. The rooster was the Messenger of Light and the Destroyer of Darkness and Demons (Rasmusson, 1990). That is why he was put on the highest point of the church tower and even today is a much appreciated silhouette as a - weathercock. However disgrace has also been his share. During some periods his ambition to gather females in a harem made him a non-appreciated symbol of lust and he lost his church tower dominion. It was even thought that fires could be put out if a living rooster was thrown into the flames.

As if this was not enough, he has been used as a fight animal too. In fact cockfights for religious reasons is thought to be the reason

for domestication (Rasmusson, 1990) in the very beginning more than 5000 years ago. This "sport" is now largely forbidden, except for parts of Asia and some Latin countries.

Splendour or not, since the introduction of industrialised animal management in the Western World at the beginning of the last century and the differentiation into specific meat and egg breeds (Appleby et al., 1992), the roosters have been made redundant. At least in the flocks of laying hens that produce eggs for consumption. There is no need for fertilisation and as the males do not grow as fast as broilers reared for meat production, the monetary return for keeping them is very low. About half of all chicks of layer breeds are males, which are killed as newly hatched.

Industrialised husbandry

As the husbandry became industrialised and intensified cages for laying hens were introduced. The cages were also meant to improve bird health by, for example, reducing the parasite load. However the battery cages hardly fulfil the behavioural and physiological needs of the hens (e.g. Brambell, 1965; Appleby, 1991; Baxter, 1994; European Commission, 1996). Following a strong public opinion there is, in many countries, a transition from cages to other systems for laying hens (European Union, 1999). This has put the focus on the specific problems that may arise in large flocks of hens. As many modern laying hybrids tend to be both aggressive and prone to feather peck this can cause severe welfare problems in all types of housing systems (e.g. Craig and Adams, 1984; Hansen, 1998; Keeling, 1994). Also fear reactions, like panic and flocking, can cause immense welfare problems as well as decreased production if the stress become chronic (Mills and Faure, 1991; Jones 1996).

Probably the selection for high egg production (e.g. Hughes and Duncan 1972) is a part of the explanation of some of the welfare problems. The problem may also be connected to the fact that eggs for consumption are produced in single-sexed flocks. This means that neither the sex composition nor the group size is natural for the hens. Furthermore, all individuals are of the same age. Practical on-farm experience is often that "males make female hens more calm".

The role of males

Experimental studies of domestic fowl (Craig and Bhagwat, 1974; Bshary and Lamprecht, 1994) support the picture of the male being socially dominant and of having an inhibiting influence on the agonistic behaviour among the females. However, these studies concern small groups of 10-15 hens under experimental conditions. Whether there is an effect of males on female aggressiveness also in large flocks under farm conditions has not been studied previously.

When the group of fowl is searching for food and eating or dust-bathing, it is the males that watch out for predators and give the appropriate alarm call (McBride et al., 1969, Johnsen 1963, Sullivan, 1991). It is likely that males through this behaviour may also have a fear reducing effect on the flock. Even though domestication seems to have decreased the tendency to show behaviours of high energetic costs (Schütz, 2002), the behaviour of modern hybrids do not differ in qualitative respects from that of their ancestors (Wood-Gush et al., 1978).

Hens usually prefer the company of familiar birds (Keeling and Duncan, 1991; Bradshaw, 1992; Appleby and Jenner, 1993; Dawkins, 1996) and show aggression towards unfamiliar birds (Craig et al., 1969; Hughes, 1977; Zayan, 1987). Grigor and co-workers (1995) concluded that birds might try to move within a restricted area thereby minimizing the risk of meeting strangers. Some authors have found that hens in this situation tend to keep together in subgroups of well-known birds (McBride, 1964; Bölker 1987; Grigor et al., 1995). However, according to other studies, most hens in large flocks move around without any sign of subgroup formation (Hughes et al., 1974; Appleby et al., 1989; Hughes et al., 1997; Carmichael et al., 1999). It can be assumed that social factors, like rank position, restrict the bird's movements (Craig and Guhl, 1969). Gibson and co-workers (1986) for example, found that low-ranking individuals moved only over a restricted area as compared to high-ranking birds. In a later study however, Channing and co-workers (2001) saw that birds in flocks ranging from 300 to about 900 hens seemed to move as one synchronised flock.

It can be expected that the presence of males in a flock would facilitate the formation of subgroups, which might lower aggression as individuals then stay most of the time with well-acquainted birds

and so do not have to fight strangers. This has been reported by Fölsch and co-workers (1992) in field-studies of a large number of flocks on farms in Switzerland. Widowski and Duncan (1995) reported clustering of female hens around the males in a flock of 50 females and 10 males. However, they could not identify any territorial subgroups, which was also the case in a study by Keeling and Savenije (1995).

Many of the studies referred to above concern small groups of hens under experimental conditions. Effects of males on female aggression and fearfulness in large flocks under commercial large-scale farm conditions have not been reported previously.

Aims

Domestic fowl are mainly social animals, whose behaviour do not differ much from their wild ancestors', the jungle fowl. However modern industrialised production puts a lot of constraints to and strains on their innate behaviour, which creates large welfare problems for the birds as well as economic problems for the producers. One way of creating more beneficial animal environments is to study the dynamics of social behaviour in practical large-scale on farm situations. The general aim of this thesis was to study the influence of males on female behaviour in commercial farm situations by comparing all-female to sex-mixed groups of laying hens in large flocks.

The particular aims were to answer the following questions:

- Does the reducing effect of males on female aggressiveness seen in small groups exist also in large flocks?
- Is there subgroup formation within large flocks of female hens and if so, do males enhance this formation?
- Do the females become less fearful in the presence of males?
- Does the dominance order of males influence their use of the available space and hence their contacts with the females?
- Is there attachment of females to specific males?

Material and methods

Animals, housing and methods

The effects of the presence of males on female aggressiveness and feather pecking in groups of about 500 laying hens housed in an aviary system were studied (**Paper I**). In all, ten groups of ISA Brown laying hens were observed at 21, 35, 45 and 55 weeks of age on two farms, during two consecutive years. In the second year with the second batch of birds the groups also consisted of 50 percent Shaver 288 white females. Half of the groups included one ISA Brown male per on average 24 females. Stocking-densities varied from 14.7 to 17.2 birds per m². Direct observations of 25 randomly selected focal animals per group was used. The health of the individual birds was recorded upon their arrival at the farm and at 35 and 55 weeks of age respectively as part of other projects (Gunnarsson et al. 1995). Observations of feather status, lesions and data from the post mortems of dead animals were used in this study.

Space use and aggressive behaviour was studied in flocks of 568 ± 59 ISA Brown laying hens kept in pens in an aviary system (density averaged 16 hens / m² of floor area (**Paper II**). Half of the pens contained one male per on average 24 females (mixed flocks). At peak production (36-53 weeks of age) four females roosting closely together for 14 days and four females roosting far apart from each other were taken out from each flock and put together in separate groups in small pens (1m²). Their agonistic behaviour was studied through direct observations during two days after which they were put back. This was repeated with new birds, resulting in 16 small sample groups being studied. At 70 weeks three groups of 10 females per flock roosting closely together in different parts of the pen were dyed with different colours and their locations were observed at two nights and during two days.

The effect of males was also studied in 51 flocks of laying hens (40 single-sexed and 11 mixed) in two high-density loose housing systems on 25 commercial farms in Sweden (**Paper III**). In the mixed flocks the sex ratio was on average 1 male: 350 females. This study was using data from the Swedish Agricultural Board's testing programme for evaluating new housing systems for laying hens (Algers et al., 1995; Ekstrand et al., 1997; Gunnarsson et al., 1999).

Six different hybrids were used in group sizes ranging from 250 to 5000 birds. Stocking-densities varied from 10.2 to 19.1 birds per m² floor area. Among other parameters the incidences and locations of aggressive pecks and fear reactions towards humans and novel objects were measured. Direct behaviour observations were carried out twice per flock, at 35 and 55 weeks of age.

Duration of induced tonic immobility and frequencies and duration of vigilant behaviour and transitions between behaviours of females were studied in 8 groups of 1200 LSL white laying hens each in a traditional loose-housing system on a commercial egg production farm (**Paper IV**). Half of the groups contained one male per 100 females and the density was 6.5 birds/m². Two all-female and two mixed groups were studied at the age of 41-43 weeks, and the remaining four groups were studied when the birds were 32-34 weeks old. All observations were direct observations with the observer either handling the birds (induction of tonic immobility) or standing inside the compartments among the birds. The health was also examined, following the procedure described by Gunnarsson (2000). General condition, laying status and injuries caused by aggressive pecks were especially noted.

Male dominance order, day-time location and night roost and female attachment was studied over a 30 week period in a flock of 3500 laying hens (a Swedish hybrid based on white leghorn) kept in a traditional loose-housing system at a density 7 birds per m² floor area (**Paper V**). The flock was subdivided in two groups of 1750 birds with an average male ratio of 1:190 females. One of the groups had access to an outdoor area. At ages 25, 35, 45 and 55 weeks, each male was followed for 10 minutes by the observer standing inside the animal compartment. The location and behaviour was recorded and based on number of territorial and sexual behaviours (crowings and matings) observed, a rank-order was set up which was then compared to the number of sections covered by each male. The night roost locations were also recorded. At the age of 25 weeks four males per group were randomly chosen and nine females roosting close to each male at night were marked with the same coloured leg rings as the respective males. Their night roost locations were observed at weeks 35, 45 and 55. An intensive study of male rank order and night roost and day-time location of males and females

was carried out when the birds aged 65 weeks. The health of the flock was monitored in a larger project carried out at the farm.

Statistics

Data were tested for normality according to Altman (1994). In the first study (**Paper I**) the data were then analysed using a two-way analysis of variance with year as a random effect and the effect of "males" as fixed. The SAS GLM -procedure (SAS Institute Inc., 1996a) with adequate options was used for these calculations and for the rest of the analyses (behaviour at different ages, location of feather pecks and health parameters) ANOVA (repeated-measures and one factorial) was used (Statview, 1991).

In the second study (**Paper II**) the location of the birds was analysed using chi-squared tests (Statview, 1991). Chi-squared tests were also used to test the hypothesis that hens were randomly distributed at the night roost and during the day. Differences between the groups in the frequencies of the recorded behaviours were analysed using repeated measures ANOVA (Statview, 1991). The values for day one were used to test the difference between the treatments "close" and "apart".

In the third study (**Paper III**) multiple and linear regression analyses (Statview, 1991) were used as a first step to discover correlations and to check for confounding association effects of farm, observation time etc . Unpaired data, such as the frequency of aggression in single-sexed and mixed flocks, were then analysed using a Mann-Whitney U-test (Statview, 1991).

Spearman's rank correlation test (SAS Institute Inc., 1997) was used to study possible correlations between TI and the other behaviour parameters (**Paper IV**). The effects of the independent variables on the dependent variables were analysed with the PHREG procedure in SAS (SAS Institute Inc., 1997). This procedure performs a regression analysis of data based on the Cox proportional hazards regression analysis (Altman, 1994). Due to the partly non-balanced experimental design "clustering" variables were entered into the model. Health parameters were analysed using logistic regression (SAS Institute Inc., 1996b)

Differences between high and low ranking birds in the number of sections covered were carried out using Mann Whitney U-test in the Minitab 12 for Windows (Minitab, 1998) (**Paper V**). Correlation with rank points was tested by Pearson's correlation analysis (Altman, 1994) and differences in night roosts were tested by Chi-square test.

Results

Agonistic behaviour and feather pecking in single-sexed and mixed groups of laying hens. (Paper I)

The results showed that aggressive behaviour (aggressive pecks, threats and fights) among females was significantly less frequent in groups that also included males; the mean incidence per group (\pm SE) for the four ages was for single-sexed groups: 10.1 ± 1.8 and for mixed groups: 5.9 ± 0.9 , ($P < 0.01$). Agonistic behaviour (aggressive behaviour and avoidances) among females was $63 (\pm 2) \%$ more frequent in single-sexed groups than among hens in mixed groups ($P < 0.01$). The males were seldom seen to show aggressive behaviour towards females or towards each other. For feather-pecks there were no significant differences between single-sexed and mixed groups. However there was significantly less pecking at the cloaca in the mixed groups ($P < 0.01$), but no significant difference in lesions from pecks at the cloaca.

Space use and agonistic behaviour in relation to sex composition in large flocks of laying hens. (Paper II)

The incidence of aggressive pecks during day one among birds that had been roosting close to each other was lower ($P=0.05$) than among birds that had been roosting far apart. This effect was not significant among birds from all-female flocks, but among birds from mixed flocks ($P < 0.05$). Irrespective of sex composition in the flocks, birds marked while roosting at the ends of the pens were significantly more often observed within these areas than in other areas of the pen during day time. They also came back to the same roosting sites more often at night ($P < 0.001$) than birds marked in middle areas, where the distribution in the pen in most cases did not differ from random.

Behaviour of laying hens in two types of aviary systems on 25 commercial farms in Sweden. (Paper III)

Aggression occurred in both systems mainly on the litter or in the nest areas; it did not differ between hybrids, but increased with age in the tiered system. There was no significant difference in the frequency of aggressive behaviour among hens in mixed flocks as

compared to hens in single-sexed flocks. White hybrids reacted significantly more to the keeper ($P < 0.001$) and to a novel object ($P < 0.01$) than brown hybrids.

Effects of sex composition on fear measured as tonic immobility and vigilance behaviour in large flocks of laying hens. (Paper IV)

Males had a significant effect on tonic immobility and frequency and duration of vigilance behaviour ($P < 0.001$); females in the mixed groups had shorter TI- duration and showed less and shorter vigilance than females in the all-female groups. There was no effect on the total number of behaviour transitions or on agonistic behaviours. However females in the mixed groups had less peck wounds at the comb compared to in the all-female groups (a significant effect of the males; $P < 0.05$) which indicates that the aggression level was lower.

Male dominance, space use and female attachment in large flocks of laying hens. (Paper V)

There was a significant positive correlation between male rank order and space use ($r = 0.851$, $P < 0.01$). However most males used more than half of the area and the dominance order appeared to be maintained mainly by avoidances. There was no observable attachment of females to specific males.

In summary the results of the studies are that in large flocks of laying hens;

- aggressive behaviour among females was significantly less frequent in groups that also included males (1 male:24 females), but there was no effect on feather pecking in hens housed at high stocking-densities (Paper I);
- females in mixed flocks were significantly less aggressive towards their roosting partners than towards birds roosting farther away. Irrespective of sex composition females marked in the ends of the pens were to a large extent constant in their use of space (Paper II);
- there was no significant difference in the frequency of aggressive behaviour among females in single-sexed flocks as compared to

females mixed flocks with a sex ratio of approx. 1 male:350 females (Paper III);

- females had shorter duration of tonic immobility and lower frequency and duration of vigilance behaviour in the presence of males at a ratio of 1:100 females than in all-female groups (Paper IV);
- there was a significant positive correlation between male rank order and space use in groups with sex ratios of 1 male:190 females. The females showed no observable attachment to specific males (Paper V).

General discussion

Methodological considerations

All studies in this thesis were carried out in large flocks on commercial farms. This of course has put some restrictions on the design of the studies, e.g. group sizes and stocking densities have been fixed on each farm and management routines have varied between farms. However, comparable groups were created on each farm (Paper I-IV) and experiments were carried out in these settings (Paper II and IV). Also studies of specific focal animals (stratified sampling) were carried out (Paper II and V). This approach was used to give the results general inference at the same time as a high external value.

There is always the problem of sampling enough focal animals to account for the variation in a large flock. (Altman, 1994). Bearing in mind that a too small sample may fail to detect real differences, the nomogram method proposed by Altman (1994) for deciding sample sizes of focal animals, has been used as a guide. Randomised sampling was used in for example the study of aggression (Paper I) and fearfulness (Paper IV). Here 25 birds were sampled from each group of 500 and 1200 birds respectively. In the first study the transporters randomly assigned transport crates for marking when the birds arrived at the farms. Of 50 birds marked, 25 were then randomly chosen for the studies, using a randomisation table. In the latter study the birds were picked out randomly at night in complete darkness. In the studies of small sample groups and locations (Paper II and V) stratified random samples were used (Altmann, 1974). This was also used for choosing test areas within areas containing different resources (Paper III).

Direct observations were used in all studies. In all but two of the studies (the small sample groups in paper I and the TI-induction in paper IV) the observer stood inside the animal compartments, recording the behaviour with a tape recorder. This technique has been used in a number of studies of large flocks of poultry during the last ten years. Especially when working with focal animals that move over a densely populated large area it has proven useful as it is more flexible than for example video technique. The criticism sometimes is that the presence of the observer influences the

animals' behaviour. However, after a short while (15 - 30 minutes) the birds lose interest in the human observer - still, probably, being aware of the human presence. In the present studies "settling periods" of approx. 15 minutes were used when entering an animal compartment. In fact all kinds of behaviours, including mating, were observed in the immediate vicinity of the observer.

For marking the birds numbered wing-tags were used in the first studies (Paper I and II). These could be observed at a distance and did not seem to cause the female birds any problems. The males, however, in the very first flock were pecked by the females on the wings around the tags. As a consequence, leg rings were used on the males the second year and in the following studies (Paper IV and V) they were used also for the females. This however made the birds more difficult to find and to follow than when using the wing-tags. The problem was overcome, though, as in this case the stocking-density was quite low (6.5-7 birds/m²). During the studies of night-roosting sites (Paper V) a complementary study was carried out using spray colour to mark the birds, as this was used earlier (Paper II) with satisfactory results.

Aggression was measured as frequency of aggressive behaviours (fights, aggressive pecks and threats) of focal birds either in large groups (Paper I and IV) or in small sample groups of four birds (Paper II). It was also observed in all birds moving in m² "test areas" (Paper III). Furthermore the focus of the studies varied so that in papers I, II and III the focus was on agonistic behaviour (and feather pecking) whereas in paper IV all other behaviours were recorded, the focus being on vigilant behaviour. Hence the way in which aggressive behaviour was sampled varied. However, groups with or without males were compared in each of the trials. Because of this it is probable that the results do reflect real differences, as suggested, in the ratios between males and females and possibly also stocking-densities, and not merely differences in how the behaviours were sampled.

In the present studies fear was measured by duration of tonic immobility and frequency and duration of vigilant behaviour (Paper IV) as these behaviours were thought to be influenced by the presence of males. The amount of vigilance was recorded when the focal birds were in their respective groups. To reduce handling

effects so that the TI-duration should measure the situation in the home environment (Jones, 1986; Jones, 1992; Bilčík et al., 1998), the induction was performed during the night, when the birds were roosting and easy to take out for testing. In fact only two birds out of the 200 birds that were tested, showed strong fear reactions, including vocalisation, when handled. Another factor that might have influenced the results was the possible difference between the groups regarding habituation to humans; The sex-mixed groups were all, due to the owners' decision, located towards the entrance doors. However, as the handling prior to and during TI-induction was carried out in darkness, it is not likely that possible differences in habituation to humans would have influenced the results (see also Jones, 1990; Bilčík et al., 1998).

To induce tonic immobility Jones and Faure (1981) recommend the use of a clothed cradle, because it enhances the induction and thus reduces the handling needed (repeated inductions) of the bird. However in a pilot study (Odén 1996) it was found that using a chair with a smooth and slightly hollowed seat, was just as good. Therefore such a chair was used also in the present study.

In the study of male dominance order and a possible relation to use of space (Paper V) we wanted to use the method of Jameson and co-workers (2000) where not all relationships have to be shown. However in this, as in an earlier study of well-acquainted males in large groups of laying hens (Paper I) hardly any agonistic behaviour was observed among the males. Certain males seemed to always be out of the way of the other males, resulting in a very low frequency of overt agonistic behaviour. This is in accordance with the results of Rushen (1984) who reported "spontaneous" avoidances in sexually mature flocks of fowl. As the method of setting up a rank order used by Jameson and co-workers (2000) where at least some relationships need to be established could not be used, it was decided to use crowings and matings as an alternative method of determining the dominance order. These behaviours are generally regarded as signs of high rank and have been found to correlate positively with both aggression and high rank (Rushen, 1982; Mench and Ottinger, 1991; Jones and Mench, 1991). In some of the earlier studies, however, the correlation between social rank and mating frequency have been somewhat ambiguous (Craig et al., 1977; Shabalina, 1984). This may have to do with both with experimental technique and the

background of the birds. For instance Shabalina (1984) using single, sexually inexperienced males that were tested for 5 minutes with groups of 30 females, could not show any relationship between sexual activity and dominance rank. It has been shown by Leonard (1993a) that an early experience with the opposite sex enhances mating success, as well as the opposite may decrease sexual behaviour and increase aggression (Leonard , 1993 b). In the study by Jones and Mench (1991) of male dominance rank and mating success in a multi-male flock, positive correlations between rank, frequency of matings and fertility as measured by DNA fingerprinting were found.

As the present thesis deals with social behaviour in groups of animals, statistical tests have been applied at a group level. When necessary, data have been tested for normality and when the design has been partly unbalanced, due to on farm practical reasons, models have been constructed and tests used to account for this. For example variables suspected to give a "cluster" effect have been put into a fixed model (Paper IV) so that the effect of the variable under study ("males or not males") could be correctly handled.

In the study the terms "group" and "flock" have been applied several times. In daily use these concepts may seem partly overlapping, however in these studies the following definitions were used: *Flock* - a large unit of birds of the same age and origin, located at the same farm. The flock could be subdivided into *groups* of smaller size. This meant that a unit of hens could be regarded as a "group" in one study, as it was one of more groups in a larger "flock", whereas the same group in another study could be termed a "flock", if it was subdivided further.

Discussion of the results

Domestic fowl, as their wild ancestors, the jungle fowl, inherently form small groups (Collias and Collias, 1967) with a dominance order based on individual recognition. The male controls the females and guards the group against predators (McBride et al., 1969). In large, crowded flocks where the hens cannot recognize all other flock members and cannot emigrate or spread over a larger area, the social environment for control of behaviour is probably of

importance. The findings in this study suggest some explanations of how this control works focusing on the role of the males.

Reduced aggression and fearfulness

This study has shown that the presence of males reduces female aggression also in large groups of hens (Paper I). Furthermore, females appear to be less fearful in flocks that also contain males, compared to in all-female flocks (Paper IV). This confirms the experience of many farmers, namely that keeping males in flocks of laying hens, have a beneficial impact on the female hens as it makes them calmer. Also, effects on the health of the birds have been shown by Kathle and co-workers (1996). They got a significantly impact of males both on cannibalism (less, though no difference in over-all mortality) and production (higher laying rate and egg mass, fewer mislaid eggs and higher food conversion ratio). Interestingly, they found no significant differences in these respects between sex ratios of 1:30 and 1:130. In the present study (Paper I) there was significantly less pecking at the cloaca in the mixed groups ($P < 0.01$). However there was no significant difference in prevalence of actual lesions from pecks at the cloaca. In addition Abrahamsson (1998) found significantly less peck wounds at head and comb in groups of 620 birds with a sex ratio of 1 male:100 females as compared to all-female groups. These results were confirmed in the present study (Paper IV).

The role of sex ratio and bird density

In the present study the sex ratios have been either 1:24 (Paper I and II) or 1: 100 (Paper IV), 1:190 (Paper V) or about 1:350 (Paper III), and a significant effect on aggression has been shown only at the largest sex ratio of 1:24. The reduction of aggression_by males probably works mainly through direct social dominance, and at short distances, with an enhanced subgroup formation playing a minor role, as signs of home ranges also occur in all-female flocks (Paper II). In both cases the lack of an effect on aggression with one male per 350 or more females is not surprising; one male can hardly dominate or have an impact on the behaviour with so many females effectively. An effect on fearfulness, on the other hand, was found at ratios of 1:100. The predator control function probably works over quite large distances, and the area that the birds occupied in this study was most likely well within the limits of effective vigilance

(Proctor & Broom, 2000). Moreover, the males mingled well with the females at the ratio of 1:190 (Paper V).

Other considerations that must be made is the health of the birds at very high or very low sex ratios. It is known from practice and also scientifically studied (Campo and Davila, 2002) that keeping one male per hen, is more stressful than at ratios of 1:3, 1:5 and 1:11, shown by higher heterophil to lymphocyte ratios, for the females. Whereas the contrary - keeping too few males - can be detrimental to the males in large flocks (Paper III, Odén and Algers, 1996). To get optimal "male effects" both on behaviour and health of the females - as well as the males - the sex ratio in large flocks shall probably not be lower than about 1:150 and not higher than 1:10. The latter is the sex ratio frequently used in breeder flocks to ensure fertilization at the same time as good health of the birds is maintained.

Another possible factor that might influence any effect of males in large flocks is the bird density. So far, however, research on density has dealt mostly with production or reproductive success and mostly in cage. Aggression may be connected to stocking-density and group size. Laying hens kept in small groups in cages have been shown to increase their aggressive behaviour with increased group size (Craig et al., 1969; Al-Rawi and Craig, 1975). Concerning density, a curvilinear relationship has been found. Generally, aggression increases until a peak at approximately 800 cm² per bird and then overt aggression seem to decline (Polley et al., 1974; Al-Rawi and Craig, 1975; Craig, 1982). A probable explanation put forward by Ylander and Craig (1980) is that in very crowded environments the socially dominant individuals are extremely close to the subordinates, which inhibits the aggressive behaviour of the latter. Instead, the subordinates show more submissive behaviours (Algers et al., 1984). The densities used in this study have all been in the range of those used commercially: 16 birds /m² (Paper I and II), 9-19 birds /m² (Paper III), 6.5 birds /m² and 7.0 birds /m² (Paper IV-V). As different hybrids also have been used, and these often differ in both frequency and intensity of agonistic behaviour (e.g. Marsteller et al., 1980), a comparison is not relevant regarding for example aggression levels. However, to examine the effect of males more closely, further studies on the interaction between sex ratio and density are needed.

Subgroup formation and individual recognition

The higher incidence of aggression in groups of birds roosting far away from each other than in groups of birds that roosted closely together in this study (Paper II) indicate that birds roosting closely were acquainted to each other while those roosting far apart were not. The difference was significant in groups from mixed flocks, which implicates that males have an impact, possibly by enhanced subgroup formation. However, the results could also reflect a rebound effect of the males' reduction of female aggressiveness towards strangers.

The results (Paper II) provide evidence for the existence of home ranges as most birds tended to prefer the area where they had their night roost - at least if it was easily localised (see below). These results are therefore indications of the existence of subgroup formation in large flocks. In another of the studies (Paper V) there was no apparent female attachment to certain males. Furthermore the females seemed to be more attached to specific areas than to the males, both during day- and nighttime, and all animals moved around a lot. This does not support the hypothesis of the males as enhancers of subgroup formation in large flocks.

In order to identify a conspecific a hen has to come quite close (Dawkins, 1995). In small groups this would result in dominance relationships based on individual recognition, whereas in larger groups of hens a system depending on status signalling rather than recognition of individuals seems likely to appear as suggested by Pagel and Dawkins (1997), later supported by findings of D'Eath and Keeling (1998). Subgroup formation in large flocks is, according to this theory, rather unlikely to occur as it would cost too much, since the limiting factor is the cost and pay off for the fights it takes to establish a peck order. The theory explains high levels of aggression in large groups as being fights over resources with little or no individual recognition, but recognition of signals of dominance and subordination. However, the limit where hierarchy formation is not based on individual recognition is not known.

McBride and Foenander (1962) studied "subgroups" of 80 birds, Appleby and Jenner (1993) used subgroups of 40, while Widowski and Duncan (1995) and Keeling and Savenije (1995) could find no

clear sign of a further subgroup formation within groups of 60 and 70 birds respectively. However in all these groups the birds probably could recognise each other individually so that a further division into subgroups was not necessary.

Based on the study of groups of four birds taken out from the larger flocks it can not be concluded how large or how firm each group of acquainted birds was, though the results indicate that subgroups are formed. In fact, a hen's preferred group size is still not known. Lindberg and Nicol (1996) concluded that a hen's group size preference probably is influenced by its position in the hierarchy, and also that space seems to play an important role. Probably the group-size must be well over 100 birds for subgroup formation to occur. Studies by Guhl (1953), who found evidence of a peck-order in a flock of 96 hens, by Craig and Guhl (1969), reporting territoriality (a sign of group formation) in single-sexed flocks of 400 laying hens and by McLean et al. (1986), who reported uneven space use by individual birds in a flock of about 1200 hens, confirm this assumption. However Hughes et al. (1997) reported a lack of social structure in flocks of 300 birds. These contradicting findings could be related both to methodology (e.g. the way the birds were selected) and to the fact that it may be difficult to discover signs of smaller groups in a large flock in a crowded intensive system (Paper II). It is for instance shown, in paper II, that birds mingle more during the day than at the night-roost.

Furthermore, it is not unlikely that a strive to form subgroups exists at the same time as a system based on status signalling is working, were senders/receivers are not individually identified. There is clearly a need for all birds to react properly (to avoid aggression) to less or non familiar birds (from other subgroups) when encountered.

In large crowded, uniform flocks perhaps a "subgroup" would perhaps be best defined as a group of acquainted birds where the individual bird feels safe at night or when resting at any time, while during most of the day-time the birds, independent of their "subgroup", or simply because they cannot manage to keep together, use more of the total environment where they also run into less well-known birds. Males might both strengthen the

”reassurance” effects while resting at night as well as during the day-time act more like ”highway patrollers” that are spotting trouble and reacting quickly to it. Male intervention in female aggression has been observed in mammals (primates) (Ehardt and Bernstein, 1992), in fish (Walter and Trillmich, 1994), but also in birds (Davies, 1992). In the present study male dominance hierarchy in large flocks of acquainted birds appeared to be maintained mainly by avoidances (Paper I and V) which means that they do not contribute to a high degree to the aggression in the flock. This confirms earlier observations by for instance Guhl and co-workers (1945). In a large mixed flock both subgroup formation, status signalling and male supervision might all contribute to lower the level of aggressiveness, the latter probably being the most effective.

The idea to use the TI-reaction in the present study (Paper IV) was based on the assumption that also domesticated hens in large flocks indoors show this strongly innate behaviour due to the evolution of successful anti-predation reactions (Gallup, 1977; Rovee-Collier et al., 1983). However, as discussed by Bilčík and Keeling (1998) who found increased TI duration with increasing group size, in these situations the TI might also reflect fearfulness of the other hens in the flock, as competition increases with increased flock size. Possibly the longer TI-reaction could also reflect reduced recognition of other hens in large flocks. Hence the males, rather than reducing the females' fear of a "predator", reduce their fear of other flock mates (Paper IV). This is in accordance with the finding that the males reduce the aggression between female hens (Paper I). It is also in accordance with the lower levels of vigilance in mixed groups in this study (Paper IV), as this behaviour may reflect the monitoring of not only predators activity, but also the activities of other group members, as discussed by Elgar (1989).

Localisation

Just like other birds, hens probably use visual cues to localise themselves. As shown by Collias et al (1966) hens in a natural habitat may have difficulties if they cannot rely on well-known environmental cues. They found that red jungle fowl birds that had been moved about 400 meters from their home territory had great difficulties in finding their way back. In an intensive housing system each section is quite similar to the other, except for the end sections with the limiting walls. In this light the results of the present study

are logical; birds roosting near the ends of the system are more constant in their use of space, simply because localisation is easier for them, than for birds roosting in the central part of the system. It is therefore highly probable that subgroup formation is facilitated by physical structures, such as pen walls, which may help the birds to localise themselves, as earlier suggested by McBride and Foenander (1962). This was also concluded by Appleby et al. (1986) in a study of nesting behaviour in a commercial flock of 4000 broiler breeders. A further aspect is that males might be used by females as environmental clues. However in the present studies (Paper V) the males seemed to move around more than the females and there was no observable attachment of females to specific males.

In another study, Appleby et al (1989) found that the birds moved around and used most of the area available, but that some birds (35-65%) used the area unevenly and appeared to have home-ranges. There are also other studies that have given similar results (e.g. Hughes et al. 1974; Gibson et al. , 1986; McLean et al., 1986). However none of these authors have drawn any conclusions about possible subgroups. In their studies they have used hens marked as individuals and not as groups. Looking at the results in paper II, it may well be that the groups marked at the ends of the pens (about 66% of all marked birds) are the ones that are able to find their way home, while the rest of the marked birds (those 33% marked in the centre of the pens) have difficulties and so are the ones that appear to be all over the pen at random. The different results from different studies or flocks within studies, might then simply stem from the fact that the birds were marked at random, which was not the case in the present study, where stratified sampling was used. The results of paper V, that birds are rather inconsistent in their choice of roosting location, may have been caused by disturbances at night (when checking the birds' roosting sites) and the fact that in this study the house consisted of 14 sections. In paper II the number of sections were only four or five, depending on the compartments, which may have facilitated localisation.

Practical implications

To reduce fear in flocks of fowl a number of methods have been suggested and used (Jones 1996). Enrichment of the environment is one of these which has been shown to have beneficial effects

especially when applied from an early age of the birds (Reed et al., 1993). Especially novel objects as well as humans have been used (Jones, 1996). Newberry (1995) criticised many of the enrichment efforts for being too much based on human centred judgements and not always biologically relevant for the animals. Following this line more suitable enrichment should include creating 1) for fowl socially relevant groups and 2) providing biologically functioning environments making localisation possible and providing birds with possibilities to seek protection. Moreover there are indications of environmental complexity reducing frequency of aggressions in large groups of domestic fowl (Estevez et al., 1998).

Laying hens of today experience a prolonged breeding season. Introducing males under these circumstances must be very relevant in terms of social behaviour. As the males also have a beneficial effect on aggression and fear and a positive effect on health and production, this solution may also have economic advantages for the production, as shown by Kathle and co-workers (1996). Furthermore this complies with the welfare demands on animal husbandry systems put forward by Fraser (1993) of "high biological functioning, freedom from suffering and positive experiences" for the animals as well as "the quality of life" definition by Mench (1998) in the discussion of how animal welfare work can be brought further.

Based on the findings in this study, practical applications in order to reduce aggression and fear in large flocks of hens producing eggs for consumption might be to include enough males in the flocks. To get optimal effects both regarding bird health, behaviour and production the sex ratio should probably be in the range of 1 male per 50 -150 females. Furthermore, in order to be able to handle a large number of females and to avoid spread of contagious disease, it is crucial that the males come from the same hatch as the hens as well as being brought up together with them. A further action could be make the flocks smaller (100-200 hens) and/or to provide environmental features to make it easier for the birds to localise themselves.

Egg quality is often said to be at risk if males are introduced into flocks producing eggs for human consumption. In fact there is a risk that fertilised eggs will be downgraded when sold for human

consumption within the European Union (European Commission, 1991). However in a study of the quality of fertilised versus non-fertilised eggs (Odén, 1996) that had been stored for a month at + 5° C and at + 15° C, no statistically significant difference was found.

The males will inevitably consume feed, and this has to be taken into account when discussing the introduction of males. Kathle and co-workers (1996) who used a small Norwegian breed of males calculated that the extra feed consumed by the males was 0.8 grams per hen per day at a sex ratio of 1:130. At the higher ratio of 1:30 the extra feed consumption was calculated to be 3.5 grams per hen per day, hence a difference of 2.7 grams.

However before introducing males in a larger scale into laying flocks, parameters such as sex ratio and density effects need to be studied further. Other factors to investigate include breed and working environment, as the males of different layer breeds may differ in the interaction with females (Kathle et al., 1996) and sometimes interact with stockpersons (von Wachenfeldt, personal communication, 2002).

Conclusions

The results in this thesis show in conclusion that;

- males reduced female aggressiveness also in large groups of laying hens;
- females were relatively constant in their use of space and were, in mixed groups, less aggressive towards roosting partners than towards other females, hence showing signs of subgroup formation and an effect of males;
- females were less fearful in the presence of males than in all-female flocks as measured by the duration of tonic immobility and the frequency and duration of vigilance behaviour;
- there was a positive correlation between male dominance rank and use of space. However also lower ranking males used much of the available space and mingled with the females;
- there was no observable female attachment to specific males.

In addition it may also be concluded that for domestic fowl, especially in large flocks in extensive stables, environmental features seem to be of importance to facilitate localisation. Furthermore, it appears that the male effect on female aggressiveness acts mainly through direct social dominance and that sex ratios probably play a major role for this function.

Sammanfattning

Intresset för inhysningssystem där hönsen hålls frigående har ökat i takt med att bursystemet har kritiserats på djurskyddsmässiga grunder. Dagens moderna hönshybrider som är avlade för hög äggproduktion har dock ofta visat sig vara både aggressiva och benägna att plocka fjädrar av varandra, vilket kan leda till stora djurskyddsproblem. Förmodligen hänger problemen delvis samman med att hönorna hålls i likåldriga, enkönade, mycket stora flockar. En erfarenhet många djurägare har, är att tuppar i flocken gör hönorna lugnare. Tuppar hålls dock vanligen inte i värphönsflockar vid produktion av konsumtionsägg.

Höns bildar naturligt rangordningar. I en stor flock där en höna inte kan känna igen alla andra, utan ständigt möter mer eller mindre okända individer, kan man förvänta sig att aggressionsnivån ökar, eftersom höns oftast föredrar välkända gruppmedlemmar och bemöter okända med aggressioner. Det finns tecken som tyder på att höns i stora flockar strävar efter att bilda mindre grupper inom den större flocken, men det finns också studier där det verkar som om hönsen i stora flockar rör sig utan någon social gruppstruktur alls och huvudsakligen reagerar på statussignaler snarare än på individuell igenkänning.

Hönsens vilda släktingar, de röda djungelhönsen, lever i grupper ledda av den ranghögste tuppen som även försvarar reviret under fortplantningsperioden. Tuppen befruktar sina hönor och skyddar dem mot rovdjur. Studier av små grupper på 10-15 domesticerade höns har visat att tuppen är socialt dominant och har en aggressionsdämpande effekt på hönorna. Om denna effekt finns även i kommersiell produktion i stora flockar har däremot inte studerats tidigare. Inte heller om tuppars närvaro leder till att hönorna blir lugnare och mindre rädda. Målet med denna avhandling var därför att ta reda på om det är mindre aggressioner och lugnare hönor i stora hönsflockar i kommersiell skala med tuppar i flocken än utan. Vidare, att studera hur tupparna utövar sitt eventuella inflytande över hönorna, om t ex bildning av undergrupper i en större flock sker, och hur denna påverkan hänger samman med tupparnas inbördes dominansförhållanden.

I ett voljärsystem med ca 16 hönor per m² studerades effekten på aggressiva beteenden och även fjäderplockning vid en relativt hög andel tuppar (1 per 24 hönor, i flockstorlekar på ca 500 höns) (Delarbete I). Som en jämförelse studerades även flockar med en mycket låg andel tuppar (1 per ca 350 hönor, i flockstorlekar varierande från 250 till 5000 höns, mellan ca 10-19 hönor per m² och med varierande hybrider i två olika golvsystem) (Delarbete III). I flockarna med 500 höns och hög andel tuppar studerades även var märkta grupper av höns uppehöll sig i voljären under dagtid, samt var de sov på natten. Förekomsten av aggressiva beteenden hos hönor som sovit nära varandra i flocken jämfört med hönor som sovit långt från varandra studerades också (Delarbete II). För att ta reda på om hönorna också blev lugnare då tuppar fanns med, studerades hur länge hönor ”spelar” döda, (tonisk immobilitet; ju längre - desto räddare) i flockar på vardera 1.200 djur med 1 tupp per 100 hönor i hälften av flockarna. Också mängden spaningsbeteenden (vilken kan antas vara mindre om hönorna är lugnare) mättes. Studierna skedde i ett traditionellt golvsystem med 6.5 hönor per m² (Delarbete IV). Förhållandet mellan tuppars inbördes rang och hur de utnyttjar tillgänglig yta, samt om de knyter hönor till sig studerades i en flock på 3.500 höns i ett golvsystem med 7 höns per m² och med 1 tupp per 190 hönor (Delarbete V). Alla studier skedde genom direkta observationer av fokaldjur.

Det visades att tuppar hade en aggressiondämpande effekt på hönorna också i stora flockar, men att andelen tuppar måste vara tillräckligt stor, eftersom ingen effekt sågs då andelen tuppar var liten. Ingen effekt på fjäderplockning sågs. Studierna visade också en effekt av tuppar på bildning av undergrupper då hönor i flockar med tuppar var mindre aggressiva mot de hönor de sovit nära på natten än mot dem som de sovit långt ifrån. Hönor som sov i ändarna av voljären, var mer konstanta när det gällde både sovplats och vistelse dagtid än dem som var märkta i mitten av voljären. Förmodligen är därför en miljö där hönsen lätt kan hitta av betydelse för deras förmåga att bilda undergrupper. Såväl signalering av status (då ”okända” höns möts) som bildandet av undergrupper och direkt social dominans från tupparnas sida, bidrar förmodligen till att sänka aggressionsnivån i stora flockar.

Tuppar påverkade även rädslonivån hos hönorna. I könsblandade grupper hade hönorna kortare tonisk immobilitet och mindre mängd spaningsbeteenden än i grupper utan tuppar. Högrankade tuppar använde mer av utrymmet än de som stod lägre i rang, även om också dessa använde en stor del av utrymmet. Någon koppling mellan individuella tuppar och hönor kunde inte påvisas.

Några av slutsatserna som kan dras av studien är, att närvaron av tuppar gör hönor mindre aggressiva och även mindre rädda också i stora flockar i intensiv produktion. Förmodligen har tupparna inverkan främst genom direkt social dominans över hönorna, men även genom att de kan underlätta bildandet av undergrupper, vilket dock verkar vara beroende av en miljö som hönsen lätt kan orientera sig i. Effekten av tuppar på aggressiviteten i stora flockar blir mindre vid lägre andel tuppar och verkar också vara en funktion av tupparnas inbördes dominansordning, då högrankade tuppar rör sig mer bland hönorna.

För att minska förekomsten av aggressiva beteenden och göra hönorna lugnare, och därmed också minska risken för skador, bör det vara positivt att ta med ett tillräckligt antal tuppar i flockarna, d v s 1 tupp till ca 50 - 150 hönor. Av smittskyddsskäl och för att tupparna ska ha lämplig erfarenhet, måste de komma från samma kläckning och vara uppfödda tillsammans med hönorna. En ytterligare åtgärd kan vara att minska flockstorlekarna till 100-200 djur och/eller göra miljön lättare att orientera sig i för hönsen.

Inom EU nedgraderas ofta befruktade ägg. Lagras äggen svalt (+5°C - +15°C) är risken för en kvalitetsskillnad visavi obefruktade ägg liten. Tupparna förbrukar ofrånkomligen extra foder. En norsk studie har dock visat på moderata ökningar (0.8 g/dag/höna vid en könskvot på 1:130). Innan könsblandade flockar generellt kan rekommenderas som standard för värphönsflockar bör dock effekter av olika könskvoter och beläggningsgrader undersökas närmare. Likaså bör faktorer som hybridskillnader och arbetsmiljö studeras ytterligare, då dessa kan tänkas påverka hur tupparna fungerar med hönorna, men också med skötarna.

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